

Attorney Docket No.: 44352-0010-00-US

Application No.: 10/588,140

Office Action Dated: December 28, 2007

Reply Dated: March 19, 2008

### **AMENDMENTS TO THE SPECIFICATION**

Please delete the present Sequence Listing and replace it with the substitute paper copy of the Sequence Listing filed herewith. Please renumber the pages of the specification accordingly.

Please delete the paragraphs on page 4, lines 4-12 and replace them with the following paragraphs:

In accordance with an aspect of the present invention, there are provided a protein, comprising an amino acid sequence of ~~SEQ. ID. NO. 1~~ SEQ ID NO: 1, which has the activity of hydrolyzing dextran, starch, mutan, inulin and levan, a derivative thereof, or a fragment thereof.

In accordance with another aspect of the present invention, there is provided a gene of ~~SEQ. ID. NO. 2~~ SEQ ID NO: 2, encoding the protein, the derivative or the fragment, a derivative thereof, or a fragment thereof.

Please delete the paragraph on page 5, lines 1-5 and replace it with the following paragraph:

FIG. 1 shows an amino acid sequence (SEQ ID NO: 1) of the carbohydrolase derived from *Lipomyces starkeyi* (LSD1) according to the present invention and a 2052 bp nucleotide sequence (SEQ ID NO: 2) encoding the amino acid sequence, wherein PCR primers for cloning the protein in a vector are underlined;

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Please delete the paragraph on page 10, line 2 to page 11, line 1 and replace it with the following paragraph:

For the first strand cDNA synthesis, reverse transcription was conducted with 0.5 g of the total RNA isolated from *L. starkeyi*, in the presence of the modified oligo-dT primer T18NN (5'-GAGAGAGAGAGAGAGAGAGAACTAGTCTCGAGTTTTTTTTTTTT-3') (SEQ ID NO: 5). 10 µl of the first strand cDNA was used to amplify a part of the base sequence coding for glycanase. A pair of the degenerated primers DC-F and DC-R was constructed with reference to seven conserved regions known in dextranase. The design of the primers DC-F (5'-ACCTGGCA(T/C)AG(A/G) (A/T/G) (A/C) (C/A)-3') (SEQ ID NO: 6) and DC-R (5'-G(G/C) (C/T) (T/G)CC(G/C)ACCTGCTT(A/G)TA-3') (SEQ ID NO: 7) was based on the peptide sequences TWHH(D/N) (N/S/T) (SEQ ID NO: 8) (conserved region I) and YKQVG(S/A) (SEQ ID NO: 9) (conserved region V), respectively. Using these primer sets, PCR was conducted to give a putative glycanase gene fragment of about 1.1 kb. The PCR product was purified from the agarose gel with the help of an AccPrep™ gel extraction kit (Bioneer, Korea), followed by the ligation of the purified DNA fragment to a pGEM-T easy vector (Promega, USA). DNA sequencing was conducted in the Korea Basic Science Institute. To obtain an intact gene for glycanase, RACE (rapid amplification of cDNA ends) was carried out on the basis of the information about the 1.1 kb DNA fragment. In this regard, 5'-RACE and 3'-RACE depended on 5'-full RACE Core Set and 3'-full RACE Core Set (both TaKaRa, Japan) so as to allow for a full size cDNA encoding glycanase. Through the 5'-RACE, a 180 bp PCR product was obtained while the 3'-RACE resulted in a 900 bp PCR product. Therefore, a glycanase gene (*lsd1*), about 2 kb long in total, was acquired.

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Please delete the paragraph on page 11, line 26 to page 12, line 22 and replace it with the following paragraph:

Using a set of the synthetic primers DX-F: 5'-GTCCCTTGAGCTCCCAAC-3' (Sequence List 3) (SEQ ID NO: 3) and DX-R: 5'-TCAACTAGAATTCATGAACTTCC-3' (Sequence List 4) (SEQ ID NO: 4), PCR was carried out in the presence of Taq DNA polymerase with 30 cycles of denaturing temperature at 94°C for 1 min, annealing temperature at 52°C for 1 min and extending temperature at 72°C for 2 min while a DNA fragment corresponding to the glycanase gene (*lsd1*) served as a template. The PCR product was ligated with a pGEM-T easy vector with which transformation was carried out. The plasmid prepared from the transformed cells was treated with EcoRI to excise the PCR product which was then ligated with a pYES2 vector (Invitrogen, USA). In this regard, the vector was previously digested with EcoRI and treated with CIAP for preventing self-ligation. The transfection of the resulting recombinant plasmid into *S. cerevisiae* was carried out with an electroporation method. Selection for transformants grown in an SC medium utilized an induction medium (2% galactose, 0.3% blue dextran, lacking uracil). When SC plates inoculated with the transformants were incubated at 30°C for two to six days, halos resulting from the hydrolysis of dextran were formed around colonies if they anchored the recombinant plasmid. Colonies around which a clear halo was formed against the blue background were selected and the clone carrying the gene of interest was named pYLSD1.